

1           1. A microparticle less than about 20 microns in  
2 diameter, comprising:  
3           a polymeric matrix;  
4           a lipid; and  
5           a nucleic acid molecule, wherein the microparticle  
6 is not encapsulated in a liposome and the microparticle does  
7 not comprise a cell.

1           2. The microparticle of claim 1, wherein the  
2 nucleic acid molecule is circular.

1           3. The microparticle of claim 1, wherein the  
2 nucleic acid is a plasmid.

1           4. The microparticle of claim 1, wherein the  
2 nucleic acid molecule comprises an expression control  
3 sequence operatively linked to a coding sequence.

1           5. The microparticle of claim 1, further comprising  
2 a targeting molecule.

1           6. The microparticle of claim 1, further comprising  
2 a stabilizer.

1           7. A preparation of microparticles comprising a  
2 plurality of the microparticles of claim 1.

1           8. A microparticle less than about 20 microns in  
2 diameter, comprising:  
3           a polymeric matrix;  
4           a lipid; and  
5           a nucleic acid molecule comprising an expression  
6 control sequence operatively linked to a coding sequence,

1 wherein the coding sequence encodes an expression product  
2 selected from the group consisting of:  
3 (a) a polypeptide at least 7 amino acids in length,  
4 having a sequence essentially identical to the sequence of  
5 (i) a fragment of a naturally-occurring mammalian protein;  
6 or (ii) a fragment of a naturally-occurring protein from an  
7 infectious agent which infects a mammal; or (iii) a  
8 plurality of the fragments of (i), linked in tandem; or (iv)  
9 a plurality of the fragments of (ii), linked in tandem;  
10 (b) a peptide having a length and sequence which  
11 permit it to bind to an MHC class I or II molecule;  
12 (c) a polypeptide consisting of at least two  
13 peptides of (b) either linked in tandem or sharing an  
14 overlapping sequence; and  
15 (d) any of (a), (b), or (c) linked to a trafficking  
16 sequence,  
17 provided that the expression product optionally  
18 includes an amino terminal methionine residue, and further  
19 provided that the expression product does not have an amino  
20 acid sequence identical to that of a full-length, naturally-  
21 occurring protein.

1 9. The microparticle of claim 8, wherein the lipid  
2 is selected from the group consisting of a cationic lipid,  
3 an anionic lipid, and a zwitterionic lipid.

1 10. The microparticle of claim 8, wherein the lipid  
2 is cetyltrimethylammonium.

1 11. The microparticle of claim 8, wherein the lipid  
2 is a phospholipid.

1           12. The microparticle of claim 8, wherein the lipid  
2 is phosphatidylcholine.

1           13. The microparticle of claim 8, further  
2 comprising a second lipid.

1           14. The microparticle of claim 8, wherein the  
2 expression product is a polypeptide consisting of at least  
3 two peptides of (b) linked in tandem, wherein the at least  
4 two peptides of (b) are not identical.

1           15. The microparticle of claim 8, wherein the  
2 expression product is a polypeptide consisting of at least  
3 two overlapping peptides of (b).

1           16. The microparticle of claim 8, wherein the  
2 expression product comprises a peptide having a length and  
3 sequence which permit it to bind an MHC class I molecule.

1           17. The microparticle of claim 8, wherein the  
2 expression product comprises a peptide having a length and  
3 sequence which permit it to bind an MHC class II molecule.

1           18. The microparticle of claim 8, wherein the  
2 expression product is immunogenic.

1           19. The microparticle of claim 14, wherein the  
2 expression product is immunogenic.

1           20. The microparticle of claim 15, wherein the  
2 expression product is immunogenic.

1           21. The microparticle of claim 16, wherein the  
2 expression product is immunogenic.

1           22. The microparticle of claim 17, wherein the  
2 expression product is immunogenic.

1           23. The microparticle of claim 8, wherein the  
2 expression product (1) has an amino acid sequence that  
3 differs by no more than 25% from the sequence of a naturally  
4 occurring peptide recognized by a T cell; and (2) is  
5 recognized by the T cell.

1           24. The microparticle of claim 8, wherein the  
2 expression product consists of an amino acid sequence at  
3 least 50% identical to the sequence of a fragment at least  
4 10 amino acids in length of a protein selected from the  
5 group consisting of myelin basic protein (MBP), proteolipid  
6 protein (PLP), invariant chain, GAD65, islet cell antigen,  
7 desmoglein,  $\alpha$ -crystallin, and  $\beta$ -crystallin, wherein the  
8 fragment binds to an MHC class II molecule.

1           25. The microparticle of claim 8, wherein the  
2 expression product comprises an amino acid sequence  
3 essentially identical to a sequence selected from the group  
4 consisting of SEQ ID NOS 1-46.

1           26. The microparticle of claim 8, wherein the  
2 expression product comprises a trafficking sequence selected  
3 from the group consisting of a sequence which trafficks to  
4 endoplasmic reticulum, a sequence which trafficks to a  
5 lysosome, a sequence which trafficks to an endosome, a  
6 sequence which trafficks to an intracellular vesicle, and a  
7 sequence which trafficks to the nucleus.

1           27. The microparticle of claim 8, wherein the  
2 expression product comprises an amino acid sequence  
3 essentially identical to the sequence of an antigenic  
4 portion of a tumor antigen.

1           28. The microparticle of claim 8, wherein the tumor  
2 antigen is selected from the group consisting of the  
3 proteins listed in Table 3.

1           29. The microparticle of claim 8, wherein the  
2 expression product comprises an amino acid sequence  
3 essentially identical to the sequence of an antigenic  
4 fragment of a protein naturally expressed by an infectious  
5 agent selected from the group consisting of a virus, a  
6 bacterium, and a parasitic eukaryote.

1           30. The microparticle of claim 29, wherein the  
2 infectious agent is selected from the group consisting of  
3 herpes simplex virus, hepatitis B virus, hepatitis C virus,  
4 *Plasmodium* species, *Chlamydia*, and mycobacteria.

1           31. The microparticle of claim 29, wherein the  
2 infectious agent is human papilloma virus.

1           32. The microparticle of claim 29, wherein the  
2 infectious agent is human immunodeficiency virus.

1           33. A preparation of microparticles comprising the  
2 microparticle of claim 8.

1           34. A method of administering a nucleic acid to an  
2 animal, comprising  
3 providing the microparticle of claim 1; and

1           introducing the microparticle into the animal.

1           35. The method of claim 34, wherein the  
2 microparticle is introduced into a mucosal tissue of the  
3 animal.

1           36. The method of claim 35, wherein the mucosal  
2 tissue is vaginal tissue.

1           37. A process for preparing microparticles,  
2 comprising:

3           (1) providing a first solution comprising a polymer  
4 dissolved in an organic solvent;

5           (2) providing a second solution comprising a  
6 nucleic acid dissolved or suspended in a polar or  
7 hydrophilic solvent;

8           (3) mixing the first and second solutions to form a  
9 first emulsion; and

10          (4) mixing the first emulsion with a third solution  
11 to form a second emulsion;

12          wherein at least one of the first, second, and third  
13 solutions comprises a lipid; and

14          wherein both mixing steps are carried out in a  
15 manner that minimizes shearing of the nucleic acid while  
16 producing microparticles having an average diameter smaller  
17 than 100 microns.

1           38. The process of claim 37, wherein the lipid is  
2 included in the first solution.

1           39. The process of claim 38, wherein the lipid is  
2 present in a concentration of 0.001 to 10% (weight/volume)  
3 in the first solution.

1           40. The process of claim 37, wherein the lipid is  
2 included in the second solution.

1           41. The process of claim 40, wherein the lipid is  
2 present in a concentration of 0.001 to 10% (weight/volume)  
3 in the second solution.

1           42. The process of claim 37, wherein the second  
2 solution further comprises a stabilizer compound or a  
3 surfactant.

1           43. The process of claim 37, wherein at least one  
2 of the first, second and third solutions further comprises a  
3 second lipid.

1           44. The process of claim 37, wherein the lipid is a  
2 cationic lipid.

1           45. The process of claim 44, wherein the lipid is  
2 cetyltrimethylammonium.

1           46. The process of claim 37, wherein the lipid is  
2 selected from group consisting of phosphatidylcholine,  
3 phosphatidylethanolamine, phosphatidylserine, and  
4 phosphatidylinositol.

1           47. The process of claim 46, wherein the lipid is  
2 phosphatidylcholine.

1           48. The process of claim 37, comprising the  
2 additional steps of:  
3           subjecting the microparticles to a temperature below  
4 0°C, to produce frozen microparticles; and

1 lyophilizing the frozen microparticles, to produce  
2 lyophilized microparticles.

1 49. A microparticle produced by the process of  
2 claim 38.

1 50. A microparticle produced by the process of  
2 claim 40.

1 51. A method of administering nucleic acid to an  
2 animal, comprising  
3 providing the preparation of claim 7; and  
4 introducing the preparation into the animal.